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Systematic Studies on Korean Rodents: VII. Immunological Analyses of Serum Proteins of Seven Species

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한국산 설치류의 계통분류학적 연구 : VII. 7종의 혈청단백질의 면역학적 분석

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적 요

한국에 서식하고 있는 설치류 7종(7아종)의 혈청단백질을 immunoprecipitin, immunodiffusion 및 immunoelectrophoresis 분석을 하였다. 각 종의 혈청단백질은 서로 달랐으며, 종간의 차이도 크게 나타났다. 즉 serological correspondence의 변이에 있어서, 집쥐(쥐 아목)는 99.6인데 다람쥐(다람쥐 아목)는 2.7이었다. 그리고 쥐 아목내의 쥐 과에 속하는 6종(6아종)중에서는 대륙밭쥐(갈밭쥐 아과)가 8.2로 가장 낮았다.

Key words: systematics, Korean rodents, immunotaxonomy

INTRODUCTION

Development in the areas of chemo-, immuno-, cyto-, and numerical taxonomy are enormous, and there have been a conflict between molecular biologists and morphologists about the merits of their data (Maxon & Wilson, 1975; Fergusson, 1980), but modern molecular techniques have not yet pushed comparative morphology into the shadows (Patterson, 1987). Crovello (1969) advocated that a classification should be the product of all available characters distributed as widely and evenly as possible over the organisms studied.

The simplest and most venerable technique for extracting comparative information from molecules is immunology, and there is a positive correlation between serological cross-reactivity and similarity in the amino acid sequence (Cristofolini, 1980). Systematically oriented comparisons have been made of serum proteins of wood mice, *Apodemus* ssp., (Fraguedakis-Tsolis *et al.*, 1983) and of albumin of bats, Phyllostomidae (Honeycutt & Sarich, 1987).

Woon (1967) noted that 14 species of rodents are inhabited in Korea, and Koh (1989) confirmed that 11 species of them are living in South Korea. Jones & Johnson (1965) reviewed taxonomically Korean rodents on the basis of the description of type specimens, and Koh (1989) performed morphometric analyses with 26 external and cranial characters of 24 species of Korean mammals, including 11 species of rodents. Chromosomal and morphometric comparisons were carried out with two species of the genus *Apodemus* in Korea (Koh 1988) and ultrastructures of spermatozoa in six species of Korean rodents were compared (Yang, 1989).

Here we present the patterns of serum proteins in seven species (seven subspecies) of Korean rodents by both quantitative immunoprecipitin and qualitative (immunodiffusion and immunoelectrophoresis) techniques.

MATERIALS AND METHODS

All 24 samples comprising seven species of Korean rodents were trapped and used [*Rattus norvegicus caraco* Pallas from Chongju, 4; *Rattus rattus tanezumi* Temminck from Chongju, 3; *Mus musculus mollosinus* Temminck from Chongju, 4; *Apodemus agrarius coreae* Thomas from Mt. Weolak, 3; *Apodemus peninsulae peninsulae* Thomas from Mt. Weolak, 3; *Clethrionomys rufocanus regulus* Thomas from Mt. Weolak, 3; *Tamias sibircus asiaticus* Thomas from Mt. Weolak, 4].

Individual blood sera of samples were prepared by heart puncture. Antisera against laboratory rat (*Rattus norvegicus*, Sprague-Dawley) were produced in rabbits (New Zealand white) as follows (Sarich & Wilson, 1966): Two injections of 0.5ml serum, emulsified with 0.5ml complete Freund's adjuvant, were given subcutaneously to each rabbit's back for three months at 1 week interval. The rabbits were bled 6 days after the fifth to the last injection.

Immunodiffusion was carried out by the method of Garvey *et al.* (1977). Gels were prepared using agarose in Barbital-tris buffer, pH 8.8. Double diffusion was performed in gels supported on 9 × 8 (cm) glass plate at room temperature for 24 hours. For immunoelectrophorsis Williams' method (1960) was modified. Troughs and wells were punched and each well contained sample of antigen. Electrophoresis was carried out at 90 mA for 2 hours, and a sample of antiserum was pipetted to each trough, and then plate was incubated in a moist box at room temperature.

The quantitative analyses of precipitin was conducted by the modification of Prager & Wilson (1971). To 0.25ml of 0.9 per cent NaCl containing antigen diluted in series was added 0.25ml of undiluted serum. The tubes were incubated at 37°C for 24 hours and then centrifuged in the cold for 30 min. at 2600 rpm. The precipitates were washed twice with 2ml of cold 0.9 per cent NaCl, and were dissolved in 3ml of 0.5M NaOH, and then the absorbance at 280nm was read. The percentage of precipitation (serological correspondence) is derived from the calculation: (sum of per cent absorbance for the heterologous test / that for the homologous test) × 100.

RESULTS

In double immunodiffusion test shown in Fig. 1 there were five archs (3 dark, 2 faint) in homologous reaction, *; five archs (3 dark, 2 faint) in *Rattus norvegicus caraco*, C; five archs (3 dark, 2 faint) in *Rattus*

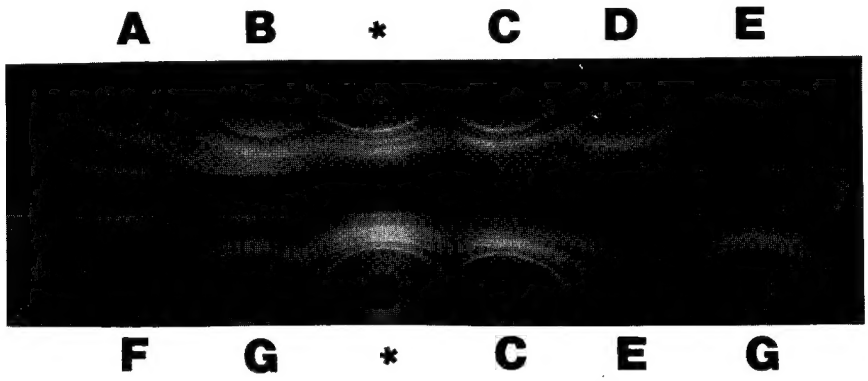

Fig. 1. Immunodiffusion patterns of serum proteins. Seven species of Korean rodents are *Mus musculus mollosinus*, A, *Rattus rattus tanezumi*; B, *Rattus norvegicus caraco*; C, *Apodemus peninsulae peninsulae*; D, *Tamias sibircus asiaticus*; E, *Clethrionomys rufocanus regulus*; F, and *Apodemus agrarius corease*; G, * indicates homologous reaction with laboratory rat. N, P, and I mean non-identity, partial identity, and complete identity, respectively.

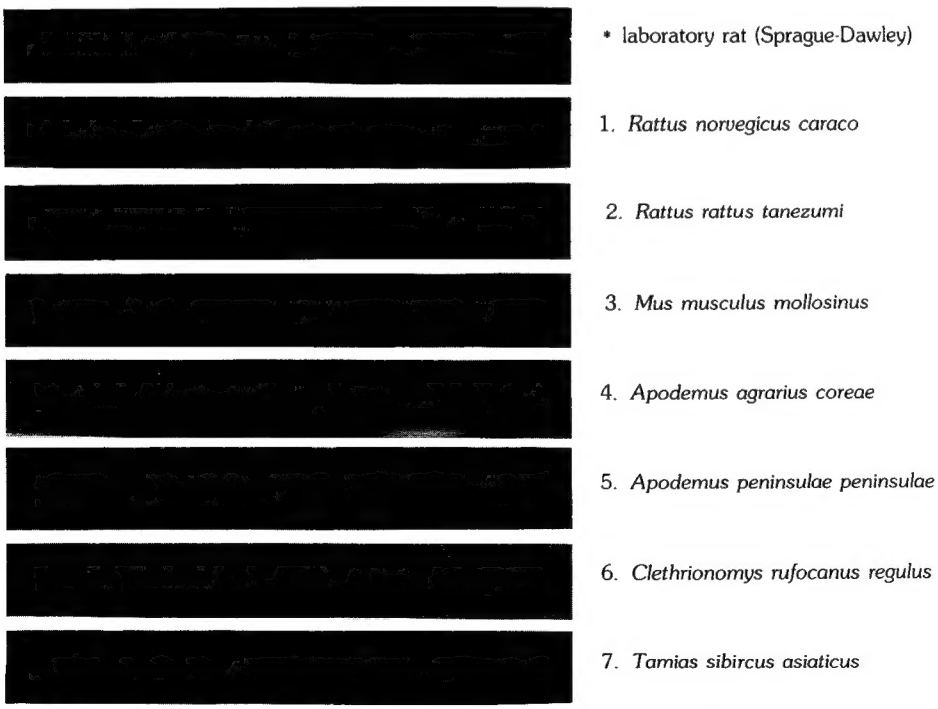

Fig. 2. Immunoelectophoretic patterns of serum proteins in seven species of Korean rodents. * indicates homologous reaction.

rattus tanezumi, B; four archs (1 dark, 3 faint) in *Mus musculus mollosinus*, A; three archs (2 dark, 1 faint) in *Apodemus peninsulae peninsulae*, D; three archs (2 dark, 1 faint) in *Apodemus agrarius coreae*, G; one faint arch in *Clethrionomys rufocanus regulus*, F; and one faintest arch in *Tamias sibircus asiaticus*, E. Among the reactions resulted in five archs, non-identify (N) in one of two faint archs, partial identity (P) in one of three dark archs, and complete identity (I) in other three archs were revealed between homologous reaction (*) and *Ratus rattus tanezumi* (B), whereas complete identity (I) was shown in all five archs between homologous reaction (*) and *Rattus norvegicus caraco* (A).

By immunoelectrophoresis (Fig. 2), ten pricipitin archs were revealed in homologous reaction (*); ten archs in *Rattus norvegicus caraco;* nine in *Rattus rattus tanezumi;* five in *Mus musculus mollosinus;* five in *Apodemus agrarius coreae;* four in *Apodemus peninsulae peninsulae;* one in *Clethrionomys rufocanus regulus;* and no in *Tamias sibircus asiaticus.*

Distinct antigenic differences among the serum proteins of seven species of Korean rodents by quantitative immunoprecipitin tests were shown and summarized in Fig. 3 and Table 1. Per cent absorbance and serological correspondence were 75.6 and 100 in homologous reaction, 75.3 and 99.6 in *Rattus norvegicus caraco*, 69.7 and 92.2 in *Rattus rattus tanezumi*, 25.0 and 33.1 in *Mus musculus mollosinus*,

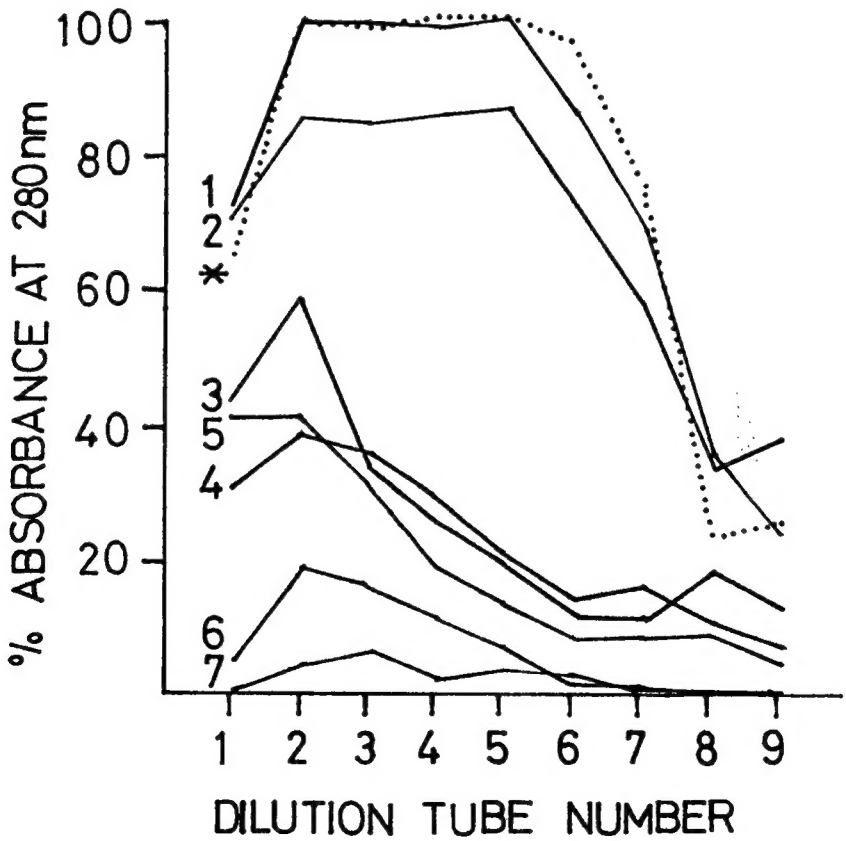

Fig. 3. Whole immunoprecipitin curves of serum proteins showing per cent absorbance readings obtained with increasing antigen dilution. * and numerals indicate homologous reaction and seven species of Korean rodents respectively (for species names see Fig. 2).

Table 1. Quantitative immunoprecipitation of sera in seven species of Korean rodents. * indicates homologous reaction.

Species	Mean % absorbance at 280 nm	Serological correspondence
* laboratory rat (Sprague-Dawley)	75.6	100.0
Rattus norvegicus caraco	75.3	99.6
Rattus rattus tanezumi	69.7	92.2
Mus musculus mollosinus	25.0	33.1
Apodemus agrarius coreae	19.5	25.8
Apodemus peninsulae peninsulae	17.7	23.4
Clethrionomys rufocanus regulus	6.2	8.2
Tamias sibircus asiaticus	2.0	2.7

19.5 and 25.8 in *Apodemus agrarius coreae*, 17.7 and 23.4 in *Apodemus peninsulae peninsulae*, 6.2 and 8.2 in *Clethrionomys rufocanus regulus*, and 2.0 and 2.7 in *Tamias sibircus asiaticus*, respectively.

In summary, the results of three tests were found to be concordant (see Figs. 1, 2, and 3 and Table 1) and a distinct pattern was revealed in each species. Moreover, antigenic divergence among the seven species of Korean rodents was appeared to be enromous. *Tamias sibircus asiaticus* of the suborder Sciuromorpha is the most distantly related with other six species (family Muridae) of the suborder Myomorpha. In the Myomorpha, *Clethrionomys rufocanus regulus* of the subfamily Microtinae is distantly related with two species of the genus *Rattus* (subfamily Murinae) and somewhat diverged with three species of the genera *Apodemus* and *Mus* (subfamily Murinae). *Rattus norvegicus caraco* is the most closely related with *Rattus rattus tanezumi* and *Apodemus agrarius coreae* is closely related with *Apodemus peninsulae peninsulae: Mus musculus mollosinus* is more closely related with two species of the genus *Apodemus* than two species of the genus *Rattus*.

DISCUSSION

Prager & Wilson (1971) noted that precipitin analysis is more useful than micro-complement fixation technique in giving one a rapid measure of cross-reactivity and that strong correlation was observed between the results of the two immunological tests. Simon (1969) stated that there is a notable agreement between the serological correspondence as measured by the quantitative pricipitin technique of Boyden and the number of identical archs as seen in double immunodiffusion and immunoelectrophoretic plates. In this study the results based on three methods mentioned above are appeared to be concordant (see Figs. 1, 2, and 3 and Table 1) and specific patterns were revealed, indicating that each species has a different structure of serum proteins.

Double immunodiffusion has a much lower capacity to detect small sequential difference (Bobak *et al.*, 1983): 0 to 10% difference practically means identity and only those proteins which differ by more than 20% may be differentiated. Prager & Wilson (1971) stated that it seems a general rule that native proteins differing by more than 30 to 40% in sequence fail to cross-react in direct precipitin test. In *Tamias sibircus asiaticus* there were little or no cross-reaction by immunodiffusion and immunoelectrophoresis and quite

low value of serological correspondence, indicating that serum proteins between this species (suborder Sciuromorpha) and *Rattus norvegicus caraco* (suborder Myomorpha) diverged enormously.

The suborder Sciuromorpha is different from the suborder Myomorpha in the shape of infraorbital canal and the zygomatic arch (Vaughan, 1986) and in the shape of sperm head (Matano *et al.*, 1976). McKenna (1987) noted that comparison of results from various disciplines as remote as palaeontology and protein sequencing are seem to yield essentially the same phylogenetic relationships. Honeycutt & Sarich (1987) concluded that evolutionary relationships indicated by the immunological data show a high level of congruence with those suggested by both anatomical and chromosomal studies on the family Phyllostomidae. The taxonomic problems on the giant panda's phylogenetic position among the arctoid carnivores was solved by both anatomical and serological studies (Sarich, 1973).

The genus *Rattus* is a large and confusing one composed of 61 species (Corbet & Hill, 1986). Schwarz & Schwarz (1967) included in *Rattus rattus* many forms, including norvegicus, but Corbet (1978) treated *norvegicus* as a good species because of their coexistance in certain regions. Furthermore, Brown & Simpson (1981) reported that interspecific differences in sequence divergence values of mitochondrial DNA were higher than any intraspecific differences, ranging from 13.7 to 18.4%, and they concluded that *Rattus norvegicus* has decended from a lineage separated from that of any *Rattus rattus* subspecies. In the present study with the serum protiens *Rattus norvegicus caraco* is different from *Rattus rattus tanezumi* (see Figs. 1, 2, and 3 and Table 1).

The genus *Apodemus* confined to the Palaearctic region is composed of 11 species and *Apodemus agrarius* is the sole member of the subgenus *Apodemus*, which has a dorsal stripe. Apodemus peninsulae is a member of the subgenus *Sylvaemus* without the dorsal stripe (Corbet, 1978). These two subgenera are also different in karyotypes (Gagia *et al.*, 1985). *Apodemus agrarius coreae* and *Apodemus peninsulae peninsulae* in Korea differ with each other only in the length of principal plus end piece, i.e., one of eight characters in the ultrastructure of spermatozoa (Yang, 1989), and they are different slightly in immunological tests of the present study (see Figs. 1, 2, and 3 and Table 1), indicating that organisms and their molecules can evolve at independent rates, as noted by Maxon & Wilson (1975). However, Fraguedakis-Tsolis *et al.* (1983) concluded that the immunological findings on the taxonomic relationships among the three species of the genus *Apodemus* are in accordance with the morphological relationships of these species.

Whole serum reactions are usually mostly albumin reactions, but albumin immunological distance units are approximately equivalent to one amino acid substitution (Prager & Wilson, 1971), and it is necessary for further studies to use albumin.

ABSTRACT

The patterns of serum proteins in seven species of Korean rodents were analyzed by immunoprecipitin, immunodiffusion, and immunoelectrophoresis. It is found that the serum proteins of each species were different with one another and that antigenic divergence among the seven species seems to be enormous, i.e., serological correspondence ranged from 99.6 in *Rattus norvegicus caraco* (suborder Myomorpha) to 2.7 in *Tamias sibircus asiaticus* (suborder Sciuromorpha). In the six species of the family Muridae (suborder Myomorpha), the lowest value of 8.2 was shown in *Clethrionomys rufocanus regulus* (subfamily Microtinae).

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